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Use of T7 gene 6 exonuclease and phosphorothioated primers for tl manipulation of HIV-1 infectious clones.

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A method is described for the efficient substitution, deletion or insertion of any desired DNA sequence into any viral infectious clones without the limitation of naturally occurring restriction sites. The technique employs the polymerase chain reaction combined with the resistance of 2'-deoxynucleotides 5'-O-(1-thiotriphosphate) dNTPs [S] bonds (phosphorothiate bonds) to the 5'-3' double strand specific T7 gene 6 exonuclease (T7 Exo) digestion. Primers used to amplify the DNA target regions being manipulated present three phosphorothioate bonds from the fifteenth base at the 5' end. The enzyme activity was shown to be completely inhibited by the presence of more than one phosphorothioate residue at the 5' end of the DNA molecules. When the amplification products are submitted t the exonuclease digestion the hydrolytic T7 Exo activity generates a short single strand DNA tail which contains the nucleotide integrity of the 3' strand. Since the ends of two independently amplified products overlap they can regenerate a stable recombinant structure when further combined in the same reaction tube in the presence of T4 DNA ligase. This new method can be used for manipulating an HIV-1 full-length clone belonging to subtype D replacing the env (gp120) gene for an F subtype sequence.

PMID: 9672139 [PubMed - indexed for MEDLINE]

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1: Nucleic Acids Res 1978 Nov;5(11):4245-4261

T7 gene 6 exonuclease has an RNase H activity.

Shinozaki K, Okazaki T.

T7 gene 6 exonuclease has been shown to have an RNase H activity as well as a double-strand specific DNase activity by the following experiments: The RNase H activity coelutes with the DNase activity from DEAE-cellulose, phosphocellulose, hydroxyapatite, and Sephadex G-200 columns. Gene 6 exonuclease specified by a strain with a temperature sensitive mutation in gene 6 has an extremely heat-labile RNase H activity as well as a heat-labile DNase activity. T7 gene 6 exonuclease degrades the RNA region of a poly(A) . poly(dT) hybrid polymer exonucleolyticall from the 5' terminus, releasing a ribonucleoside 5'-monophosphate product. When the RNA strand of a 0X174 RNA . DNA hybrid molecule synthesized with E. coli RNA polymerase is degraded, a ribonucleoside triphosphate is produced from the 5'-triphosphate terminus. Participation of T7 gene 6 exonuclease in the removal of primer RNA in discontinuous replication of T7 DNA is discussed.

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1: J Biol Chem 1983 Sep 25;258(18):11165-11173 Related Articles, Bool

Two forms of the DNA polymerase of bacteriophage T7.

Engler MJ, Lechner RL, Richardson CC.

The DNA polymerase induced by bacteriophage T7 can be isolated in two differen forms. The distinguishing properties are: 1) the specific activities of the associated to 5' single- and double-stranded DNA exonuclease activities, 2) the ability to catalyze DNA synthesis and strand displacement at nicks, and 3) the degree of stimulation of DNA synthesis on nicked, duplex DNAs by the gene 4 protein of phage T7. Form I is obtained when purification is carried out in the absence of EDTA while Form II is obtained if all purification steps are carried out in the presence of 0.1 mM EDTA. Form I has low levels of both exonuclease activities, l than 5% of those of Form II. Form I can initiate DNA synthesis at nicks leading to strand displacement, a consequence of which is its ability to be stimulated manyfol by the helicase activity of gene 4 protein on nicked, duplex templates. On the othe hand, Form II cannot initiate synthesis at nicks even in the presence of gene 4 protein. In keeping with its higher exonuclease activities, Form II of T7 DNA polymerase has higher turnover of nucleotides activity (5-fold higher than Form I) and exhibits greater fidelity of nucleotide incorporation, as indicated by the rate of incorporation of 2-aminopurine deoxynucleoside monophosphate. Both forms of T DNA polymerase exhibit higher fidelity of nucleotide incorporation than bacteriophage T4 DNA polymerase. In the absence of EDTA or in the presence of FeSO4 or CaCl2, Form II irreversibly converts to Form I. The physical difference between the two forms is not known. No difference in molecular weight can be detected between the corresponding subunits of each form of T7 DNA polymerase measured by gel electrophoresis in the presence of sodium dodecyl sulfate.

PMID: 6411726 [PubMed - indexed for MEDLINE]